

Supporting Information

SARS coronavirus papain-like protease induces Egr-1-dependent up-regulation of TGF- β 1 via ROS/p38 MAPK/STAT3 pathway

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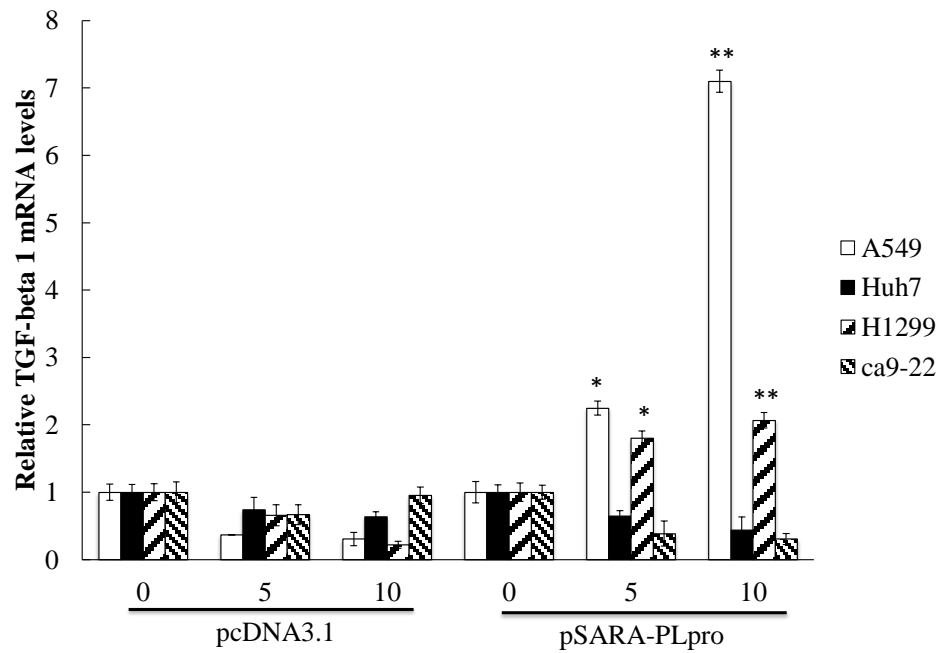
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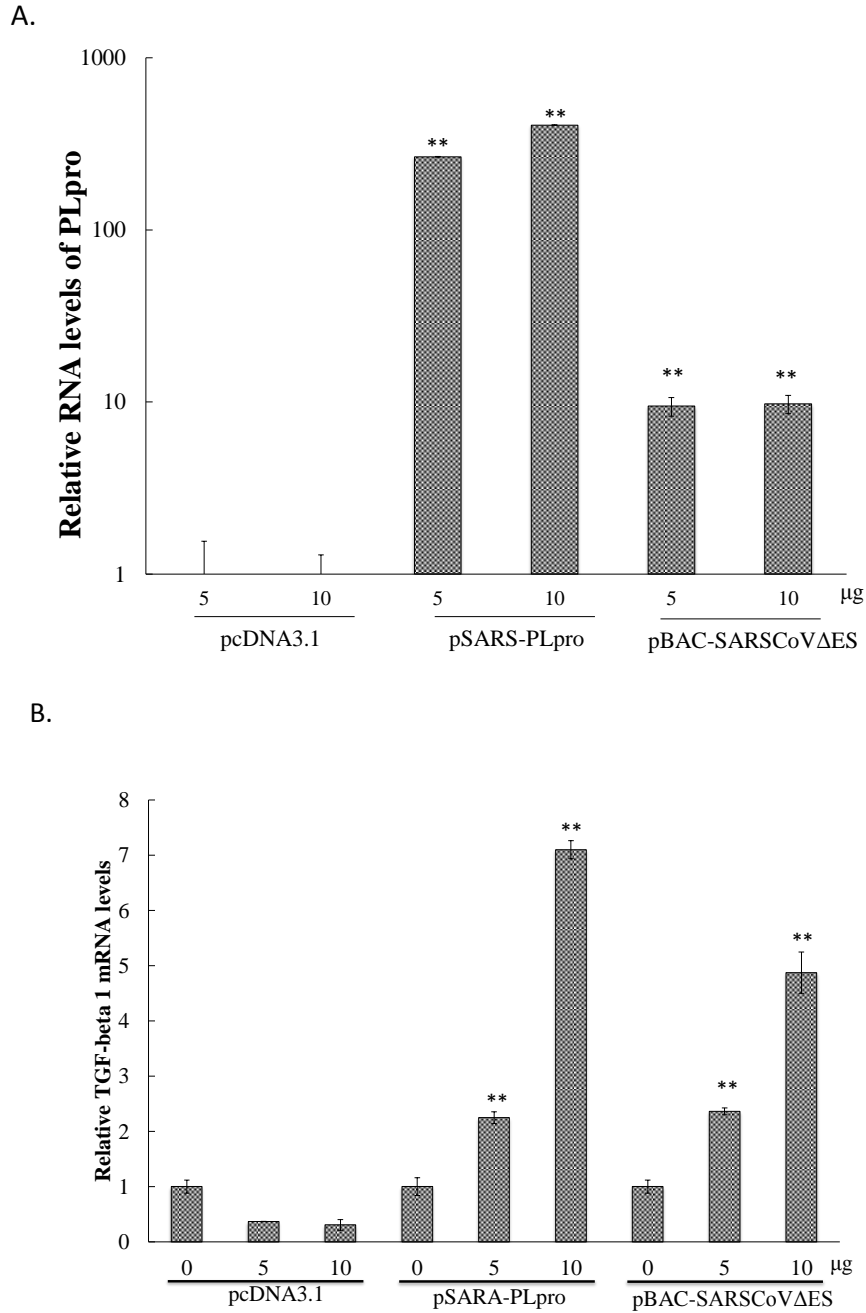
Beside A549 cells, three other cell lines Huh7 (human hepatocarcinoma), H1299 (human non-small cell lung carcinoma), and ca9-22 (human oral cancer) were used as the control for examining the TGF- β 1 induction of SARS-CoV PLpro in different cell lines (Supplemental Fig. 1). Real-time RT PCR analysis of transfected cells with pSARS-PLpro indicated that a lower level of TGF- β 1 mRNA was detected in transfected H1299 cells compared to transfected A549 cells, but no significant level was found in transfected Huh7 and ca9-22 cells.

To examine with the mRNA levels of PLpro and TGF- β 1 in the transfected cells with virus infected cells, the non-infectious SARS-CoV replicon pBAC-SARSCoV Δ ES was transfected into A549 cells to mimic the infected cells. Comparison of the expression levels of PLpro and TGF- β 1 among transfected cells with empty vector, pSARS-PLpro, and pBAC-SARSCoV Δ ES (a non-infectious SARS-CoV replicon) was further performed (Supplemental Fig. 2). The expression level of PLpro in transfected cells with pSARS-PLpro was 25-fold higher than the cells transfected with pBAC-SARSCoV Δ ES. A dose-dependent increase of TGF- β 1 mRNA levels in A549 cells was induced by pSARS-PLpro and pBAC-SARSCoV Δ ES, respectively.

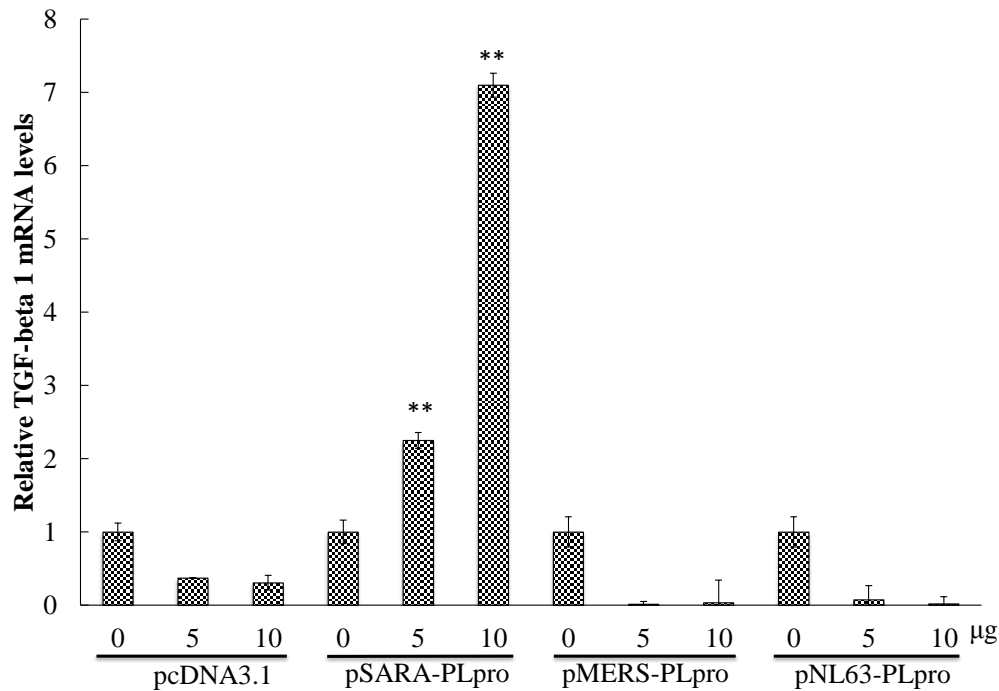
To test the specificity of SARS-CoV PLpro on TGF- β 1 induction, MER-CoV and HCoV NL63 PLpro genes were cloned into pcDNA3.1 and then measured their ability of TGF- β 1 induction (Supplemental Fig. 3). Interestingly, only SARS-CoV PLpro, but not ERS-CoV and HCoV NL63 PLpro, dose-dependently up-regulated the mRNA expression of TGF- β 1.



Supplemental Fig. 1. Relative mRNA levels of TGF- β 1 in transfected different cell lines with vector control and pSARS-PLpro. A549 (human alveolar basal epithelial), Huh7 (human hepatocarcinoma), H1299 (human non-small cell lung carcinoma), and ca9-22 (human oral cancer) cells were transiently transfected with pcDNA3.1 or pSARS-PLpro. Total RNAs of transfected cells were extracted 1-2 days after transfection. Relative TGF- β 1 mRNA level was measured by quantitative real-time PCR, normalized by GAPDH mRNA, and then presented as the relative ratio. *, p value < 0.05; **, p value < 0.01 compared with vector control cells.



Supplemental Fig. 2. Comparison of SARS-CoV PLpro and TGF- β 1 expression levels in A549 cells transfected with pcDNA3.1, pSARS-PLpro, pSARS-PLpro, or pBAC-SARSCoV Δ ES. The non-infectious SARS-CoV replicon pBAC-SARSCoV Δ ES was kindly provided by Drs. Zheng-Li Shi and Luis Enjuanes. A549 cells were transiently transfected with empty vector, pSARS-PLpro, or pBAC-SARSCoV Δ ES 2 days post transfection, harvested for total RNA extraction. The relative levels of SARS-CoV PLpro (A) and TGF- β 1 (B) mRNA level are measured by quantitative real time PCR, normalized by GAPDH mRNA, and then presented as the relative ratio. *, p value < 0.05; ** p value < 0.01 compared with vector control cells.



Supplemental Fig. 3. Relative mRNA levels of TGF- β 1 in A549 cells transfected with pcDNA3.1, pSARS-PLpro, pMERS-PLpro, and pNL63-PLpro. MERS-CoV and HCoV NL63 PLpro genes were amplified using PCR from the MERS-CoV and HCoV NL63 genome cDNAs that were provided by Dr. Chien-Te K. Tseng and Dr. Lia van der Hoek, respectively. PCR products were cloned into the vector pcDNA3.1. Total RNAs of indicated transfected cells were extracted 1-2 days after transfection. Relative TGF- β 1 mRNA level was measured by quantitative real-time PCR, normalized by GAPDH mRNA, and then presented as the relative ratio. **, p value < 0.01 compared with vector control cells.